# Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

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# SUPPLEMENTARY APPENDIX

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**Title:**

**Hospital Outbreak of Middle East Respiratory Syndrome Coronavirus (MERS-CoV)**

## CONTENTS

**Title:**

# Hospital Outbreak of Middle East Respiratory Syndrome Coronavirus (MERS-CoV)

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## METHODS

### Laboratory Methods/Sequencing

Full genome coronavirus sequences (Al-Hasa_1_2013 through Al-Hasa_4_2013) were obtained as previously described [1]. Briefly, nucleic acid was extracted from 200 µl of patient tracheal aspirate using the Roche Magnapure system following the manufacturer's protocol, the final nucleic acid was eluted in 50 µl and stored at -80 °C until use. For each sample, 1 µl of this nucleic acid was used in fifteen, 20 µl reverse transcription reactions with primers placed at 2-3 kb intervals across the 30 kb coronavirus genome, 5 µl of the resulting cDNA was then amplified in 25 µl PCR reactions. The amplicon products from each sample were pooled, individually barcoded and sequenced with Illumina MiSeq, the resulting raw 150 nt reads, approximately 1.5 million reads per sample, were processed to remove terminal primer sequences, trimmed to a median PHRED quality score of 35 and minimum length of 125 nt and assembled into full genomes using *de novo* assembly with SPAdes [2]. Assemblies were validated using reference-based assembly with SMALT v0.7.4 [3]. The open reading frames of the novel genomes (not shown) and a comparison of nucleotide changes relative to the closest existing MERS genome (England2_HPA [4]) were analyzed (Figure 4a) using Python scripts.

These full-length genomes were combined with the 5 previous published MERS genomes (KC776174, JX869059, KC667074, EMC/Munich/AbuDhabi/2013, and England2), and aligned using MEGA5. A second alignment was created by trimming the genome to include only the coding regions (ORF1ab, S, ORF3, ORF4a, ORF4b, 5, E, M, N). Maximum likelihood phylogenies were inferred from the whole-genome sequence alignment using PhyML v3.0 under a GTR+Γ substitution model, and bootstrapped 1000 times to assess the confidence in the tree topology. Further time-resolved phylogenetic trees were obtained from the concatenated coding alignment using BEAST v1.7.5. The likelihoods of runs under different models were compared, and a maximum clade credibility (MCC) tree used to summarize the most likely model (GTR+Γ substitution model, uncorrelated exponential molecular clock, and exponential population growth).

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## AUTHOR ROLES AND CONTRIBUTIONS

***Authors from the Ministry of Health and University College London (UCL) collaboration**:*
This study was initiated, designed and conducted as a major priority issue under the auspices of the research aims of Global Center for Mass Gatherings Medicine (GCMGM), Ministry of Health Kingdom of Saudi Arabia as part of ongoing collaborations with University College London (UCL). Board members of the GCMGM, Drs. Abdullah Al-Rabeeah (Chair), Ziad Memish (Vice Chair), Alimuddin Zumla and Rafaat Al- Hakkeem initiated the research on the Al-Hasa case cluster immediately after the outbreak. Dr Memish commissioned experts from USA and UK to assist. Dr. Abdullah Assiri ran the outbreak investigation for the Ministry of Health. Dr Memish as chairman of the Scientific Advisory committee of GCMGM played a critical lead role in managing, directing, funding, coordinating, and oversight of all aspects of the study and writing. Dr Zumla coordinated the genome sequencing and collation of all inputs. All MoH staff/Saudi authors played important roles in patient management, data collection and management. Drs Memish and Zumla provided guidance and insights throughout the investigation, coordinating clinical, laboratory and sequencing information and manuscript writing and submission process.

***Authors from United Kingdom:***
**From the Wellcome Trust Sanger Institute**: Drs Simon Watson, Matthew Cotten and Paul Kellam; **From UCL:** Drs Zumla and Kellam. All UK authors worked closely with Dr Memish and KSA Ministry of Health staff for sample collection, shipping, genome sequencing, data analysis, interpretation and rapid data dissemination via Genebank.

***Authors from USA and Canada:***
**From Canada**: Mount Sinai Hospital and Department of Laboratory Medicine and Pathobiology, University of Toronto, Ontario, Canada (Dr Allison McGeer); **From USA**: Johns Hopkins University, Baltimore, Departments of Epidemiology, Pathology and Medicine (Dr Trish Perl) and Department of Epidemiology (Dr Derek Cummings); Denver Health and University of Colorado-Denver (Dr Connie Price): Drs McGeer, Price and Perl and made a trip to KSA during the outbreak. Allison McGeer collected data for this report, and established the links between cases. Dr. Cummings performed the modelling and transmission analysis.

***Manuscript writing:***
Dr McGeer wrote the first draft of the manuscript. Drs McGeer, Perl, Price, Memish, Zumla and Cummings developed subsequent drafts with contributions from all authors. Drs Cotten, Kellam, Watson and Zumla contributed the text on genomes and sequencing. No external source was used to write the manuscript.

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## LEGENDS TO SUPPLEMENTAL FIGURES

**SUPPLEMENTAL Figure S1:**

**Location of Residence of Patients with confirmed MERS-CoV infection, Al-Hasa, KSA, April-May, 2013.**

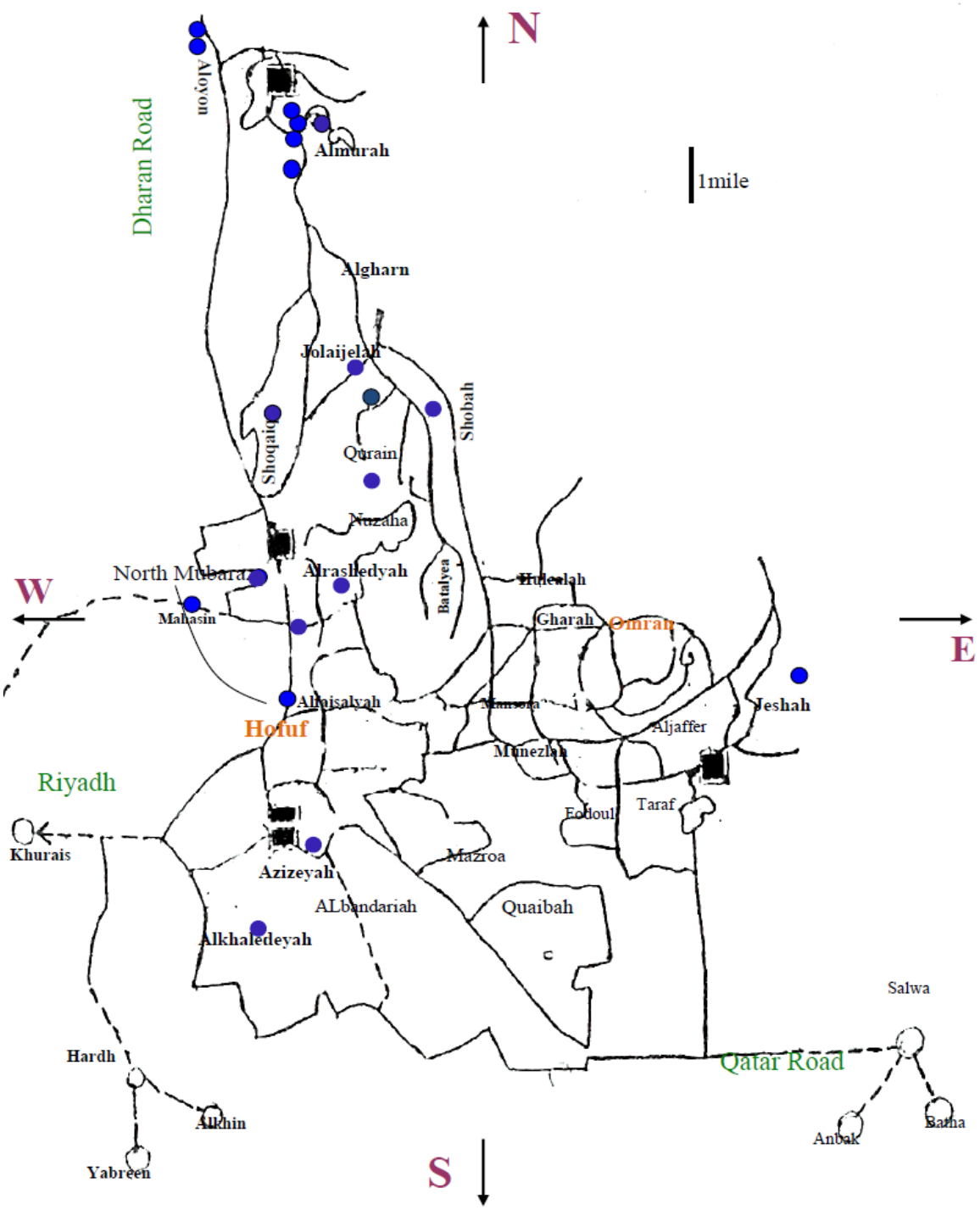
A map of the Al-Hasa region demonstrates the distribution of the 20 confirmed cases resident in Al-Hufuf and surrounding villages. Each blue dot represents one confirmed cases. Residences are distributed throughout the area, although there is an apparent cluster (composed of patients B, G, H, M, Y) in the northern village of AlMurah/AlOyoun. Three patients (transmission associated with hospital D, located 100 km to the northeast) were not residents of Al-Hufuf and cannot be displayed on map.

**SUPPLEMENTAL Figure S2:**

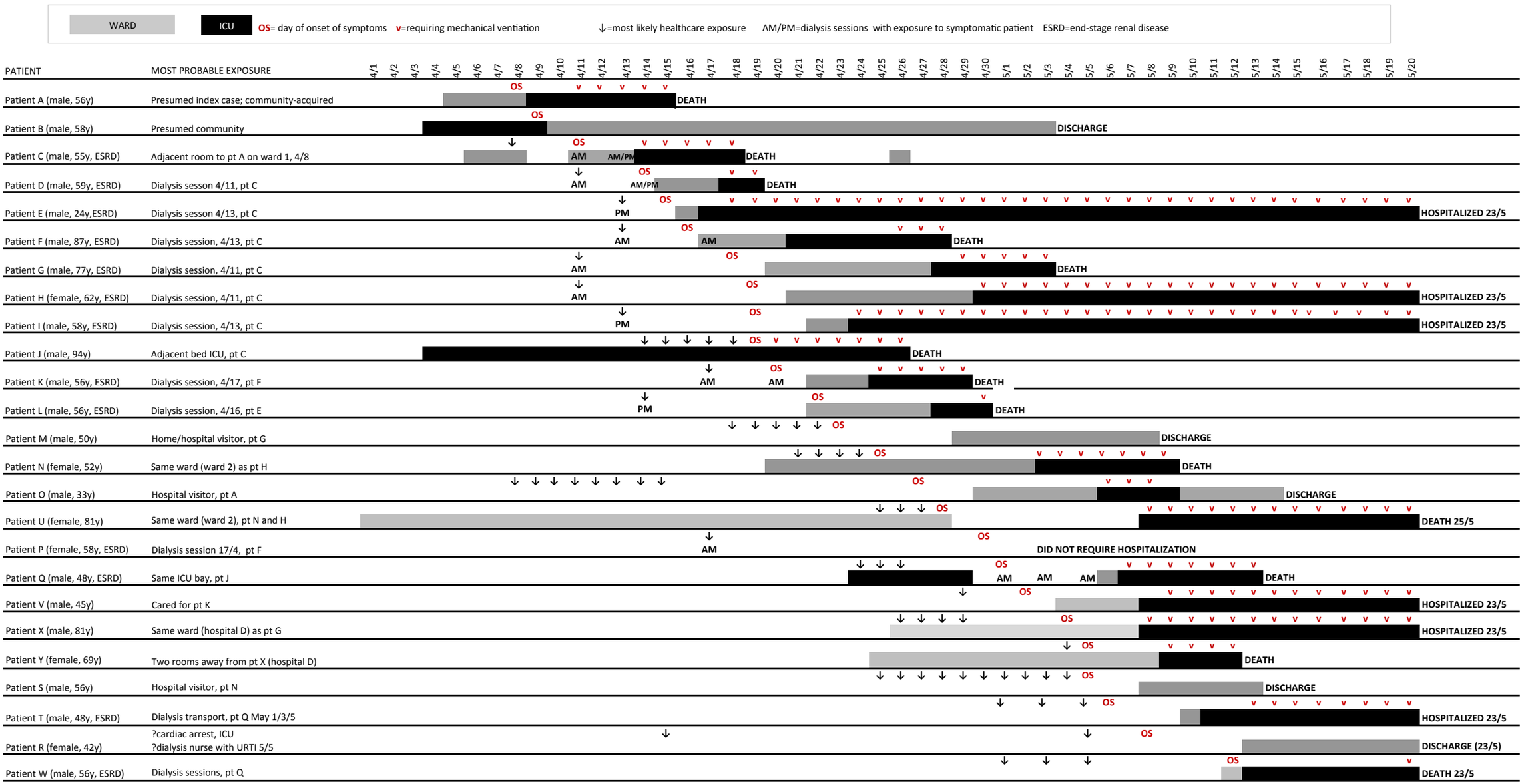
**Timeline of Events and Exposures for Patients with confirmed MERS-CoV infection, Al-Hasa, Kingdom of Saudi Arabia, April-May 23, 2013.**

Potential exposures to healthcare and clinical course are displayed for confirmed MERS-CoV infected patients, as well as the two probable cases linked to transmission events. Light gray denotes a hospital admission, dark gray represents admission to an ICU. AM/PM indicates a dialysis session in either the morning or afternoon during which transmission occurred (marked for both infector – without arrow) and infected (with downward arrow). Downward arrows represent the timing of exposure events for each patient. OS denotes symptom onset as defined in the text and V denotes mechanical ventilation; ESRD indicates that the patient was a patient with end-stage renal disease requiring hemodialysis. Outcomes as of 6 June, 2013 are noted.

**SUPPLEMENTAL Figure S1**



# SUPPLEMENTAL Figure S2



## SUPPLEMENTAL TABLE S1

### Healthcare exposures among patients with laboratory confirmed MERS-CoV, Alhasa KSA, 2013*

| Patient acquiring infection | Health care facility of transmission, if healthcare acquired | Most likely exposure in healthcare | Day(s) of illness in source patient | Other potential healthcare exposure |
|---|---|---|---|---|
| **B** | Hospital A, ward 1 | None identified | | None identified |
| **D** | Hospital A, dialysis unit | Adjacent bed, dialysis session patient C for 3.3 hours | First | None identified |
| **E** | Hospital A, dialysis unit | Adjacent bed, dialysis session, patient C for 1.0 hour | Third | Same dialysis session, patient D, 2 beds apart for 1.25 hours |
| **F** | Hospital A, dialysis unit | Adjacent bed, dialysis session, patient C for 1.3 hours | Third | None identified |
| **G** | Hospital A, dialysis unit | Same dialysis session as patient C, 3 beds apart for 3.5 hours | First | Same dialysis session, patient D, 2 beds apart for 0.75 hours |
| **H** | Hospital A, dialysis unit | Same dialysis session as patient C, 5 beds apart for 3.0 hours | First | Same dialysis session, patient D, 6 beds apart for 2.7 hours |
| **I** | Hospital A, dialysis unit | Same dialysis session as patient C, 3 beds apart for 1.6 hours | Third | None identified |
| **J** | Hospital A, ICU | Adjacent ICU bed, patient C for 3 days | Third-fifth | Same ICU as patient A but not adjacent bed for 7 days |
| **K** | Hospital A, dialysis unit | Same dialysis session as patient F, 3 beds apart for 2.8 hours | Second | Same dialysis session as patient C, 4 beds apart for 4.0 hours |
| **L** | Hospital A, dialysis unit | Adjacent bed, dialysis session, patient E for 4.3 hours | Second | Adjacent bed, dialysis session, patient D for 1.3 hours |
| **M** | Hospital A, ward 1 | Visited patient G at home and in hospital (ward 1) | First –fourth | None identified |

| | | | | |
|---|---|---|---|---|
| **N** | Hospital A,<br>ward 2 | Same ward (ward 2) as patient H x 4 days | Third-seventh | None identified |
| **O** | Hospital A,<br>ward 1 or ICU | Visited patient A in hospital | First-seventh | Exposure to patient B in same ICU bay during 2 days of visits |
| **P** | Hospital A,<br>dialysis unit | Same dialysis session as patient F,<br>5 beds apart, for 4.2 hours | Second | Two different dialysis sessions with patient K, 8 beds apart for 2.5 and 3.3 hours, dialysis session with patient I, 5 beds apart for 1.0 hours |
| **Q** | Hospital A,<br>ICU | Adjacent ICU bed, patient J, for 5 days | Sixth-eighth | Same ICU as patient A but not adjacent bed, x 7 days |
| **R** | Hospital A<br>ICU | Attended cardiac arrest for patient A, for 10 minutes | Eighth | Brief encounter with febrile dialysis nurse who did not meet case definition for testing |
| **S** | Hospital A, ward 2 or<br>Hospital B | Visited patient N in hospital A and B, medical wards | First-eleventh | Other ill patients (H and U) on ward 2, hospital A |
| **T** | Hospital C,<br>dialysis center | Shared transport to dialysis with patient Q for 3 days | First, third or fifth | None identified |
| **U** | Hospital A,<br>ward 2 | Same ward (ward 2) as patient N for 4 days; patient N ambulatory | First-third | Same ward (ward 2) as patient H, x 3 days; patient H not ambulatory |
| **V** | Hospital D,<br>ICU | Cared for and inserted central line in patient K | Ninth | Face to face contact with the symptomatic but untested relative of another case patient |
| **W** | Hospital C,<br>dialysis center | Same dialysis session and room as patient Q x 3 sessions | First, third or firth | None identified |
| **X** | Hospital D,<br>ward | Same ward as patient G for 4 days | Ninth-twelfth | None identified |
| **Y** | Hospital D,<br>ward | Same ward (2 rooms away) as patient X for 1 day | First | Same ward (ward 3) as patient G, x 4 days |

ICU=intensive case unit

*Because MERS-CoV has a median incubation period of 5 days with a range that extends beyond 10 days, patients who develop infection while hospitalized may has acquired their infection either in the community of in the hospital. In this cohort of patients, Patients J and U had been hospitalized for 15 and 24 days, respectively prior to the onset of symptoms, and patient R, a healthcare worker, had not left the hospital grounds for 13 days, making community acquisition of infection very unlikely. The majority of the nine patients receiving out-patient dialysis at hospital A are also most likely to have acquired infection associated with the dialysis unit because off the statistical improbability of a attack rate of 19% in hemodialysis patients in the absence of a detected community outbreak involving other types of immunocompromised or older patients.